UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER OF PATENTS AND TRADEMARKS
Washington, D.C. 20231
www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
09/158,272	09/22/1998	VINCENTE DIAS	10806-64	3752

7590 01/02/2003

DINSMORE & SHOHL
1900 CHEMED CENTER
255 EAST FIFTH STREET
CINCINNATI, OH 45202

EXAMINER	
WOITACH, JOSEPH T	
ART UNIT	PAPER NUMBER
1632	

DATE MAILED: 01/02/2003

26

Please find below and/or attached an Office communication concerning this application or proceeding.

File

Office Action Summary

Application No.	Applicant(s)
09/158,272	Dias et al.
Examiner	**Art Unit**
Joseph Woitach	1632

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136 (a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133).
- Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1) ☒ Responsive to communication(s) filed on *Oct 17, 2002* .

2a) ☐ This action is **FINAL**. 2b) ☒ This action is non-final.

3) ☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11; 453 O.G. 213.

Disposition of Claims

4) ☒ Claim(s) *27, 28, 32, 33, 35-50, 53-57, and 59-65* is/are pending in the application.

4a) Of the above, claim(s) _____ is/are withdrawn from consideration.

5) ☐ Claim(s) _____ is/are allowed.

6) ☒ Claim(s) *27, 28, 32, 33, 35-50, 53-57, and 59-65* is/are rejected.

7) ☐ Claim(s) _____ is/are objected to.

8) ☐ Claims _____ are subject to restriction and/or election requirement.

Application Papers

9) ☐ The specification is objected to by the Examiner.

10) ☐ The drawing(s) filed on _____ is/are a) ☐ accepted or b) ☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

11) ☐ The proposed drawing correction filed on _____ is: a) ☐ approved b) ☐ disapproved by the Examiner.

If approved, corrected drawings are required in reply to this Office action.

12) ☐ The oath or declaration is objected to by the Examiner.

Priority under 35 U.S.C. §§ 119 and 120

13) ☒ Acknowledgement is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a) ☒ All b) ☐ Some* c) ☐ None of:

1. ☒ Certified copies of the priority documents have been received.
2. ☐ Certified copies of the priority documents have been received in Application No. _____ .
3. ☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

*See the attached detailed Office action for a list of the certified copies not received.

14) ☒ Acknowledgement is made of a claim for domestic priority under 35 U.S.C. § 119(e).

a) ☐ The translation of the foreign language provisional application has been received.

15) ☐ Acknowledgement is made of a claim for domestic priority under 35 U.S.C. §§ 120 and/or 121.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO-1449) Paper No(s). _____
4) ☐ Interview Summary (PTO-413) Paper No(s). _____
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other:

Continued Prosecution Application

The request filed on October 17, 2002, paper number 23, for a Continued Prosecution Application (CPA) under 37 CFR 1.53(d) based on parent Application No. 09/158,272 is acceptable and a CPA has been established. An action on the CPA follows.

DETAILED ACTION

This application filed September 22, 1998, claims benefit of provisional application 60/062,994, filed October 23, 1997, and claims priority to foreign application 9703663-6 filed October 8, 1997 in Sweden.

Applicants amendment filed October 17, 2002, paper number 24, has been received and entered. Claims 31 and 52 have been canceled. Claims 27, 28, 32, 43, 53-55 60 and 61 have been amended. Claims 64 and 65 have been added. Claims 27, 28, 32, 33, 35-50, 53-57 and 59-65 are pending and currently under examination as they are directed to methods of genetic modification in transgenic animals.

Claim Objections

The claims stand objected to because they are not specifically drawn to the elected invention of methods of genetic modification in transgenic animals for the reasons set forth in the previous office action of June 5, 2002, paper number 21.

Applicant's arguments filed Octomber 17, 2002, paper number 24, have been fully considered but they are not persuasive. Applicants note the new amendments to the claims and argue that as amended the do not read on any of the other non-elected inventions. See Applicants' amendment, bridging pages 5-6. Applicants' arguments have been fully considered but not found persuasive.

The amendments to the claims are noted. Initially, Examiner agrees that the amendment to recite "mammalian" cells has differentiated the instant claims from groups II and III. However, the present method claims only recite one step, simply "transfecting mammalian cells with prokaryotic beta recombinase and DNA sequences containing *six* sites that allow recombination activity". There are no limitations in the claim to the type of mammalian cell, the context or location of the cell, the context or location of the *six* sites, any details on providing a transgenic mammal, or how a single step of delivery is specifically related to the elected invention of methods of genetic modification in transgenic animals. The present claims do not differentiate the method from the same step which would be used for the delivery of a polynucleotide to a subject which is encompassed by Group IV, methods of gene therapy. The claims as presently amended are drawn to mediating recombination in mammalian cells for any reason, including gene therapy which was non-elected group IV, and the means for delivery of the recombinase and target sequences are not limited to methods commonly used in making or using transgenic animals.

Claim Rejections - 35 USC § 112

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Claims 27, 28, 32, 33, 35-50, 53-57 and 59-65 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for a method of mediating intramolecular recombination between two *six* sites in the genome of an isolated mammalian host cell, comprising: (a) providing an isolated mammalian host cell whose genome contains two *six* sites target sequences stably integrated in the genome of the host cell, (b) administering prokaryotic beta recombinase obtained from *Streptococcus*, wherein the administration of said beta recombinase results in the recombination between the two *six* sites, does not reasonably provide enablement for providing the two *six* sites extrachromosomally or simply integrating DNA sequences into a genome which does not comprise target *six* sites sequences, for reasons of record set forth in the previous office actions of June 5, 2002, paper number 21.

Applicant's arguments filed October 17, 2002, paper number 24, have been fully considered but they are not persuasive. Applicants note the amendments to the claims and summarize the basis of the invention encompassed by the independent claims (pages 6-8). Applicants note that the methods would be useful in the generation of transgenic animals and the manipulation of mammalian genome. With respect to the breadth of species of animals enabled, Applicants note that COS-1 cells were used in the working examples, thus demonstrates that simian cells could work within the context of the claimed invention (pages 8-9). Finally, citing

various case law, Applicants argue that the courts have maintained that a disclosure is enabling if the artisan could make and use the invention without undue experimentation (bottom of pages 9-10). See Applicants' amendment, pages 6-10. Applicants arguments have been fully considered, and found persuasive in part.

Initially, with respect to the use of beta recombinase within the breadth of any mammalian cell, Examiner agrees that the present disclosure provides evidence for enablement in mouse and simian cells. It is noted that similar more detailed working examples are also disclosed in the post filing art of Diaz *et al.* (JBC, 1999). Further, the instant specification and the post filing art of Diaz *et al.* (JBC, 2001) teach that the required host factors supplied by the mammalian cells for beta recombinase activity are HMG family members. The art teaches that the HMG proteins are a highly conserved family of proteins among all mammals analyzed and are ubiquitously expressed throughout all the cells in a given mammal. Upon reconsideration of the teachings in the present specification and knowledge in the art, Examiner agrees that since beta recombinase can use the ubiquitously expressed HMG as the required cofactor for beta recombinase activity that the broad use of the enzyme in mammalian cells would be enabled. It is also noted that post-filing art by Diaz *et al.* (JBC, 2001) teaches that other yet to be defined cellular factors besides HMG1 are capable of acting as cofactors (page 16257, summary in abstract). The claims have been amended to encompass mammalian cells, therefore, the rejection as it is drawn to the embodiment of the claims which encompass the practice of the instantly claimed method in mammalian cells is withdrawn. However, with respect to the use of target *six* sites as an extra-chromosomal DNA sequences and the use of prokaryotic beta recombinases

other than those obtained from *Streptococcus*, and for the use of the methods in a transgenic animal *in vivo*, it is maintained that the present disclosure fails to provide the necessary guidance and description to practice the method in the breadth instantly claimed.

First, with respect to the use of any prokaryotic beta recombinase, as noted in the basis of the previous rejection, the specification and the art of record (Rojo *et al.*, 1994) teach that the only prokaryotic beta recombinase known in the art is that obtained from *Streptococcus pyogenes* plasmid pSM19035. Because of the similarities between strains of *Streptococcus* Examiner would concede that the artisan could isolate beta recombinase with the same activity from other *Streptococcus* besides only *Streptococcus pyogenes* plasmid pSM19035. However, there is no evidence that beta recombinase disclosed exists in any other prokaryotic cell. To the contrary, in comparing known prokaryotic recombinases Rojo *et al.* (JMB, 1994) teach that recombinases from various phylogenetically related Tn3/Hin exist, however the recombinases and their respective activities and required cofactors of these recombinases are quite dissimilar (page 169, discussed starting at middle of first to second columns). Additionally, the specification and the art of record provide the necessary description of the sequences of the specific *six* sites target sites for use with *Streptococcus* beta recombinase, however it fails to provide any other specific or variant target sequences which are functional with *Streptococcus* beta recombinase or sequences which would be functional with potential related recombinases isolated from other prokaryotic cells. Furthermore, it is noted that the recombinases of other family members described in the art have a distinct enzymatic activity which is different from that of beta recombinase, and based on these different activities there is no expectation that other target

recombination sequences for other recombinase are interchangeable (see Rojo *et al.* JMB, 1994 and JBC1995).

Second, the specification and the art of record clearly teach that cofactors are necessary for prokaryotic beta recombinase to mediate a recombinant event. As detailed in the specification and the previous office action, the necessity of HMG1 in mammalian cells or HU and/or Hbsu from a prokaryotic source is absolute for the resolution of the recombination event catalyzed by beta recombinase. As taught by Alonso *et al.*, the Hbsu is required for the resolution and DNA inversion mediated by beta recombinase (JBC, page 938). Further the art supports that the artisan can substitute HU from *E. coli* or of mammalian HMG1 for Hbsu functions *in vitro* as a chromatin associated protein affecting recombination (Mol Bio, page 471), however in the absence of either of these three factors, recombination does not occur (JBC, page 2943). More importantly, for function and use of these target sites *in vitro* the art teaches that the DNA to be affected must be supercoiled (Diaz *et al.* page 16262, bottom of first column, JBC (2001) and Alonso *et al.* Mol Micro, (1995)). The art and the specification do not teach if these in vitro results would be extendable to conditions present in the cell, however, post filing art teaches that within the context of a cell the DNA must be supercoiled or alternatively, it must be in the context of the genome relying on undefined features and context of the chromatin structure (Diaz *et al.* page 16262, top of second column, JBC (2001)). Therefore, for the presently claimed methods to mediate recombination as directed to genetic modification in cells *in vitro*, the only context which is enabled as evidenced by the art and the present specification is where

the target *six* sites are stably integrated into the genome of the cell or if the polynucleotide which is delivered to the cell is supercoiled.

Finally, the instant specification does not provide any new or specific methodology for making or using transgenic animals, rather it relies on methods known in the art. Generally, the specification teaches the use of the claimed methodology is for the conditional knock-out or knock-in of a gene of interest in the creation of useful transgenic animals. More specifically, the present application has defined a novel function for beta recombinase in eukaryotic cells, and proposes the use of the beta recombinase in methodology previously described for different but related recombination systems such as CRE/lox and FLP/frt (see for example Wahl *et al.* US Patent 5,654,182). As discussed above, beta recombinase differs from other recombinases known and used in the art of transgenics, and neither the instant specification, nor the art of record, has resolved the many complexities involved in targeting the *six* site sequences. To the contrary, post-filing art provides new evidence that the proposed beta recombinase methodology could be practiced more broadly than disclosed in the present specification however, the post-filing art indicates that it is unknown mammalian host cell factors and unknown chromatin structures which support the recombinase activity of beta recombinase. The specification proposes generally that prokaryotic beta recombinase/*six* sites can be used in a manner similar to that used for known recombinases CRE/LOX and FLP/FRT, however, the functionality of the system must be drawn to embodiments which are enabled by the specification. In the instant case, the art teaches that the required host cofactors for recombination activity of beta recombinase are still not completely defined, nor is the requirement for specific chromatin

structure within the context of an isolated cell. The instant specification relies on the art to practice the claimed methods in an animal, however because of the great differences between the CRE and FRT recombinases and the beta recombinases, the methods known and described in the art for CRE and FRT would not simply apply to the use of beta recombinase. None of the requirements for the location of the target six sites in a genome of a cell is given, nor are the conditions for the delivery of a polynucleotide to an animal. Further, none of the conditions for the means of delivery or the required amounts of beta recombinase to be delivered to a cell in an animal to affect recombination are described. The specification relies on the art to practice the full breadth of the instantly claimed methods in an animal, however the art fails to teach the specific methods necessary to use beta recombinase, and the specification fails to provide the necessary guidance to adapt the methods used for known recombinases to be applicable to the functionally unrelated beta recombinase.

The courts have stated that reasonable correlation must exist between scope of exclusive right to patent application and scope of enablement set forth in patent application. 27 USPQ2d 1662 *Ex parte Maizel*. Scope of Enablement has been considered in view of the Wands factors (MPEP 2164.01 (a)). As set forth above, the specification relies on the art for specific methods to make and use transgenic mammals with specific recombinase activities, and the post-filing art has provided examples which would not have been predicted by the teaching in the present specification. Further, even in light of the post-filing teachings, the factors that allow for the unpredicted activities (host factors and chromatin structure) are still unknown. In view of the quantity of experimentation necessary to determine the parameters listed above, the lack of

direction or guidance provided by the specification, the absence of working examples for the demonstration or correlation to the production of transgenic animal models of any species, and the general unpredictable state of the art with respect to the generation of transgenic animals of all species, it would have required undue experimentation for one skilled in the art to make and/or use the claimed inventions as broadly claimed.

Thus, in view of the lack of guidance, working examples, breadth of the claims, the level of skill in the art and the state of the art at the time of the claimed invention was made, it would have required one of skill in the art undue experimentation to practice the invention as claimed, and therefore, the rejection is maintained.

Claims 27, 28, 32, 53 and 55 rejected under 35 U.S.C. 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention is withdrawn.

The basis of the rejection focused on the embodiments of 'specific target sequences', and that the specification and the art of record clearly teach that beta recombinase can use only the polynucleotide sequences set forth as the *six* site sequence. Amendments to the claims to encompass only the use of *six* site target sequences has obviated the basis of the rejection.

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claims 27, 28, 32, 33, 35-50, 53-57 and 59-65 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. Specifically:

Claims 27, 28, 43, 53, 55, 60, 64 and 65 are unclear and confusing in the recitation of "wherein the prokaryotic beta recombinase is capable of using host factors provided by the mammalian cells in order to mediate recombinase activity" because it is not clear if other forms of beta recombinase which do not require host factors are contemplated. Further, it is not clear whether the claims encompass a beta recombinase that does not require a host factor or if the required factor is provided by another means of delivery would be encompassed by the claims. Amending the claims to recite 'wherein in the presence of factors provided by the mammalian cells recombination occurs.' would obviate the basis of the rejection.

Claims 27, 28, 43, 53, 55, 64 and 65 are vague, unclear and incomplete. The preamble recites that the method is directed to mediating transgenic intramolecular recombination, however, it is unclear how this is related to the single method step of delivering a recombinase and target sequences. The claims appear incomplete because there is no further steps nor embodiments which relate this step to making or using a transgenic mammal. It appears that recombination occurs only within the context of the delivered DNA and it is unclear how this is related to the intended use set forth in the preamble. It is noted that claim 28 recites "in chromatin structures" however it is unclear how this is related to the delivered DNA because it the delivered DNA which has the target six sites, not the genome. Dependent claims are

included in the rejection because they do not further clarify the basis of the rejection. For example, claim 32 recites that a recombination event "is obtained", and it is unclear if then claim 27 encompasses the practice of the method where no recombination occurs. Likewise, claims 39 and 40 are unclear for the same reason. Further, it is unclear from claim 27 if the components are delivered and present in the mammalian cell how one would not get recombination, and so claim 32, 39 and 40 does not appear to further limit claim 27. Claim 33 is confusing because how more than one recombination event is generated is unclear in light of the single step recited in claim 27. Claims 41 and 42 (also claim 44) are confusing because the six sites are delivered as DNA sequences in claim 27, so it is unclear how stating that they are extrachromosomal further limits claim 27. Claim 49 is unclear because when or how the six sites are integrated is not clearly set forth in light of the fact that claim 43 delivers these sequences by transfection. Claim 28 requires that cellular factors are supplied by the host cell, so it is unclear how claim 54 further limits this requirement or if other non-chromatin proteins are specifically contemplated.

Claim 45 is vague and unclear in the recitation of "wherein the gene coding" because 'the gene' lacks sufficient antecedent basis in claim 44. Further, the language is confusing because it is unclear what is coded by said gene.

The prior art made of record and not relied upon is considered pertinent to applicant's disclosure.

WO 99/18222, DIAZ et al., filed September 22, 1998, appears to be the PCT filing of the instant application and relies on the same foreign application filed in SE.

US 2002/0123145, OW, filed July 23, 2001, teaches use of recombinase activities and specifically recites the use of beta recombinases for use in mammalian cells.

Conclusion

No claim is allowed.

Claims 27, 28, 32, 33, 35-50, 53-57 and 59-65 are free of the are of record because the art fails to teach or make obvious the use of beta recombinase in transgenic animal methodology. At the time of filing a prokaryotic Beta recombinase was isolated from a Gram-positive broad host range plasmid PSM19035, originally isolated from *S. pyogenes* and shown to require additionally host cofactors, such as Hbsu from *S. subtilis,* to affect recombination between two *six* site target sequences. Though at the time of filing beta recombinase was known in the art as a member of the resolvase/invertase family of recombinases, and other recombinases (Cre and Flp) were used in the art in the generation of transgenic animals, these recombinases are classified in the Int family of recombinases. Importantly, the Int family of recombinases do not require additional cofactors. The present specification is the first to demonstrate that other host non-prokaryotic factors, such as the mammalian cofactor HMG, are able to serve as host factors in the presence of beta recombinase and polynucleotide sequences between two *six* sites in generating an intramolecular recombination event in mammalian cells.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Joseph Woitach whose telephone number is (703)305-3732.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Deborah Reynolds, can be reached at (703)305-4051.

Any inquiry of a general nature or relating to the status of this application should be directed to the Group analyst Dianiece Jacobs whose telephone number is (703) 308-2141.

Papers related to this application may be submitted by facsimile transmission. Papers should be faxed via the PTO Fax Center located in Crystal Mall 1. The faxing of such papers must conform with the notice published in the Official Gazette, 1096 OG 30 (November 15, 1989). The CM1 Fax Center numbers are (703)308-4242 and (703)305-3014.

Joseph T. Woitach

RAM R. SHUKLA, PH.D
PATENT EXAMINER